MICROSTIMULATION OF LUMBOSACRAL SPINAL CORD-MAPPING

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6th Progress Report
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Neural Prosthesis Program

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I. Introduction

During this quarter progress was made in the following areas:

1) A manuscript has been prepared summarizing the colon microstimulation experiments. The findings summarized in this manuscript have been outlined in detail in several previous progress reports and should be submitted for publication in the next two weeks.

2) New studies were begun this quarter which extended some of our previous studies on the hindlimb motor activity generated by lumbosacral cord microstimulation. In these studies we examined complex motor activity of the hindlimb generated by microstimulation with a single electrode. The findings from these studies suggest that microstimulation of a single site in the lumbar cord can produce a lifting movement of the hindlimb involving several groups of muscles and movement at three joints - hip, knee and ankle. An abstract summarizing these findings has been submitted to the Society for Neuroscience (a copy is appended to this report) and a detailed description is also presented below.

3) During this quarter tracing studies using pseudorabies virus to determine the location and distribution of neurons and interneurons which control the internal (smooth muscle) and external (striated muscle) anal sphincters were also begun. These experiments are extensions of previous studies which examine the innervations of the proximal and distal colon. An abstract summarizing our findings on the anal sphincter has been submitted to the Society for Neuroscience and a copy is appended to this report. The findings from these studies

indicate that the S1 and rostral S2 spinal cord segments provide the innervation to the striated muscle of the external anal sphincter (EAS) via the large motoneurons located in the ventral horn of these segments. Interneurons are located in the dorsal commissure (DC) of S1, S2, and L7. The smooth muscle of the internal anal sphincter and anal canal is innervated by preganglionic neurons in the S1, S2 and the L2 to L4 segments of the spinal cord. The details of these experiments are presented below.

II. Complex Hindlimb Motor Activity Generated by Microstimulation of the Lumbar Spinal Cord.

These studies were designed to examine complex motion of the hindlimb produced by focal microstimulation of the lumbar spinal cord. Our previous studies which measured isometric torque about the knee joint suggested that microstimulation in the dorsal horn and dorsal parts of the vertical horn may produce contractions in several groups of muscles, while stimulation deep in the ventral horn activated specific muscle groups. These early studies suggested that simulation deep within the ventral horn may activate large motoneurons or their axon directly which innervates specific muscle groups. Microstimulation in the dorsal horn and dorsal parts of the vertical horn may activate, either directly or reflexly, groups of neurons or interneurons involved in more complex hindlimb movements. In these studies the hindlimbs were allowed to move freely in space. The animals were anesthetized with pentobarbital (30-35 mg/kg) and except for the hindlimb the animals were rigidly suspended in an "Eccles" type spinal frame. A dorsal laminectomy exposed the L_4 to S_3 spinal segments and roots. Fine tipped ($400\mu^2$

expose area) activated iridium electrodes were used to stimulate sites in the lumbar spinal cord at 200μ intervals along each electrode track. Movement of hindlimb was quantiated by use of small reflective marks placed at each hindlimb joint (hip, knee, and ankle) and on the foot. The motion of the hindlimb and muscle were recorded on video tape, together with a run numbers, a light emitting diode (LED) and a timer. The LED was turned on when a stimulus was applied to the spinal cord. The video was analyzed following the completion of the experiment. A video frame grabber was used to capture selected video frames into a computer. The hindlimb motion for each run was correlated with the histological and stimulus data indicating the site and parameters of stimulation. Using the reflective marks, stick-figures of the hindlimb position could be generated for each stimulus site. In our initial experiment only the left hindlimb motion was recorded and stimulation was primarily on the left half of the spinal cord. Gross visual observations were however made of activity of the right hindlimb. The amount of force generated by the hindlimb was not recorded in these initial experiments but this will be included in future studies.

Figures 1-4 show data along four electrode tracks recorded from an experiment using the methods described above. The stick figures represents the hindlimb positions at each depth along the electrodes track. The stick figure at depth 0.0 is resting position of the hindlimb (no stimulus). The figurine in upper right corner indicates the location of the electrode track for each figure. Notice the changes in hindlimb position electied by stimulation of sites 0.4 to 2.4mm from the cord surface. These movements involve change in angle about the hip, knee and ankle joints and the coordinated contraction of several groups of muscles. If the stimulus intensity is slowly increased and decreased (intensity is modulated by a sine function) the hindlimb is raised

in a smooth gradual pattern and then slowly lowered as the stimulus intensity is once again decreased (See Also Figure 5).

At sites deep in the ventral horn the leg is extended with little change in hip angle and a small change in ankle movement. The change in ankle at these deep sites are in part due to passive changes in foot position as the lower hindlimb is extended strongly.

At more medial sites of microstimulation as seen in Figure 2 (track 2) less knee, hip and ankle flexion is seen, but extension about the knee joint is still present deep in the ventral horn. At more lateral sites (Figure 3 and 4-showing response from track 3 and 4 respectively) the coordinated hindlimb lift is seen again at superficial sites in the spinal cord. Sites deep in the ventral horn produce primarily lower hindlimb extension about the knee joint.

Figure 5 shows how a smooth coordinated lifting of the hindlimb might occur with a gradual increase and decrease in stimulus intensely. In this figure (5) the stimulus intensity is modulated from 0 to100μA with a sine function having a period of 2 seconds. The leg is slowly lifted and returned to its resting position in a smooth coordinated fashion. Force is generated by the lifting of the hindlimb but its return to its resting position is mearly the reduction in stimulus intensity and gravity returning hindlimb to its resting position. We have not measured the forces generated in these experiments but they are at least adequate to lift the hindlimb and generate a motion that appears functionally to lifting and placing the foot. The downward force and stiffness may require a least second site for microstimulation to control hindlimb stiffness (extension).

Although no direct measurements were made of the contralateral limb, some uncoordinated movement was observed as the microstimulation sites approached the midline.

The contralateral hindlimb produced only uncoordinated muscle twitches while the ipsilateral limb often produced a smooth lifting motion.

These types of studies will be continued into the next quarter with measurements of hindlimb force and bilateral hindlimb activity.

III. Transynaptic Tracing Studies to Determine the Location of Neurons and Interneurons which Control Anal Sphincter Activity.

These studies were designed to determine the location and distribution of neurons and interneurons in the lumbosacral spinal cord using the transynaptic tracer pseudorabies (PRV). The location of these neurons would provide sites in the spinal cord for microstimulation studies aimed at controlling anal sphincter activity. The methods used in these studies are standard PRV tracings methods described in previous progress reports and summarized here. PRV was injected into the striated muscle of the external anal sphincter (EAS). Since the striated muscle fibers of the EAS is closely attached and partially intermingled with the smooth muscle fibers of the internal anal sphincter (IAS) and anal canal, both anal sphincters were injected in these experiments. Therefore both large motoneurons in the ventral horn and preganglionic neurons in the sacral parasympathetic nucleus (SPN) and intermedolateral grey (IML-sympathetic) were labeled with our PRV injections. Large motoneurons were labeled deep in the ventral horn of S_1 and rostral S_2 spinal cord segments; in the dorsalateral part of Onuf's nucleus (Figure 6). Parasympathetic preganglionic neurons were labeled in the SPN in the S_1 to S_3 segments and sympathetic preganglionic neurons were labeled in the IML in L_1 to L_4 segments. Interneurons were labeled in the dorsal commissure (DC) and just lateral to the central canal in the L_7 to S_3

segments. Wether these interneurons synapses with the large motoneurons of the EAS or the preganglionic neurons of the IAS is not easily determined. However, it is likely that the most rostral located (L_7 and S_1) interneurons may project to the more rostrally located motoneurons in rostral S_2 and S_1, while those interneurons in the more caudal segments may project to the preganglionic neurons in the more caudal segments S_3 and S_2. This however is only speculation. The lumbar interneurons located in the DC and near the central canal very likely project to the sympathetic preganglionic neurons in the IML.

These types of studies will continue and probably come to a conclusion in the next quarter.

Figure 1.- Stick figures of the cat hindlimb showing the changes in peak leg movement for stimulation at various depths along microelectrode tracks #1. The location of each of four tracks are shown in the spinal cord figurine in the upper right hand corner. The same format is used for Figures 1-4. An asterisk marks the particular electrode track shown in the figurine in the upper right hand corner. Notice changes in limb position and joint angle at different sites along the electrode track. Stimulus parameters 0.2 msec. Pulse duration, 40 Hz, 100μA intensity, negative first charge balanced pulses.

Figure 2.- Same as figure 1 except showing track #2.

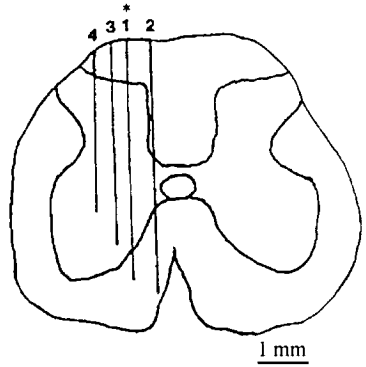
Figure 3.- Same as figure 1 except showing figure 3.

Figure 4.- Same as figure 1 except showing figure 4.

Figure 5.- Stick figure of the cat hindlimb showing changes in hindlimb movement with slow increase, followed by a slow decrease in intensity of stimulation. The stimulus intensity is modulated by a sine wave with a 2 second period. Notice gradual lifting of hindlimb with increasing intensity of stimulation and lowering of the hindlimb with decrease intensity. Stimulus parameters: 0.2 msec. pulses duration, 40 Hz, 0-100μA intensity (modulated by sine wave - peak intensity at 1 sec.).

Figure 6.- Camera lucida drawings showing the distribution of PRV labeled neurons and interneurons in the L_7 to S_2 spinal cord following injection of PRV into anal sphincter. Both the EAS and IAS were injected because of the close proximity of the striated and smooth muscle. L_7c to S_2c are shown in transverse section. The labeled neurons deep in the ventral horn of the S_1c section are large motoneurons in Onuf's nucleus. Afferent labeling in the superficial laminae of the dorsal horn are also seen as well as preganglionic neurons in the SPN. Labeling in DC and near central canal are sphincter interneurons. Bar at bottom 500μ for all figures. Three section superimposed for each level.

MS#77, Track#1, depth 0.4mm to 4.8mm, No.1-23



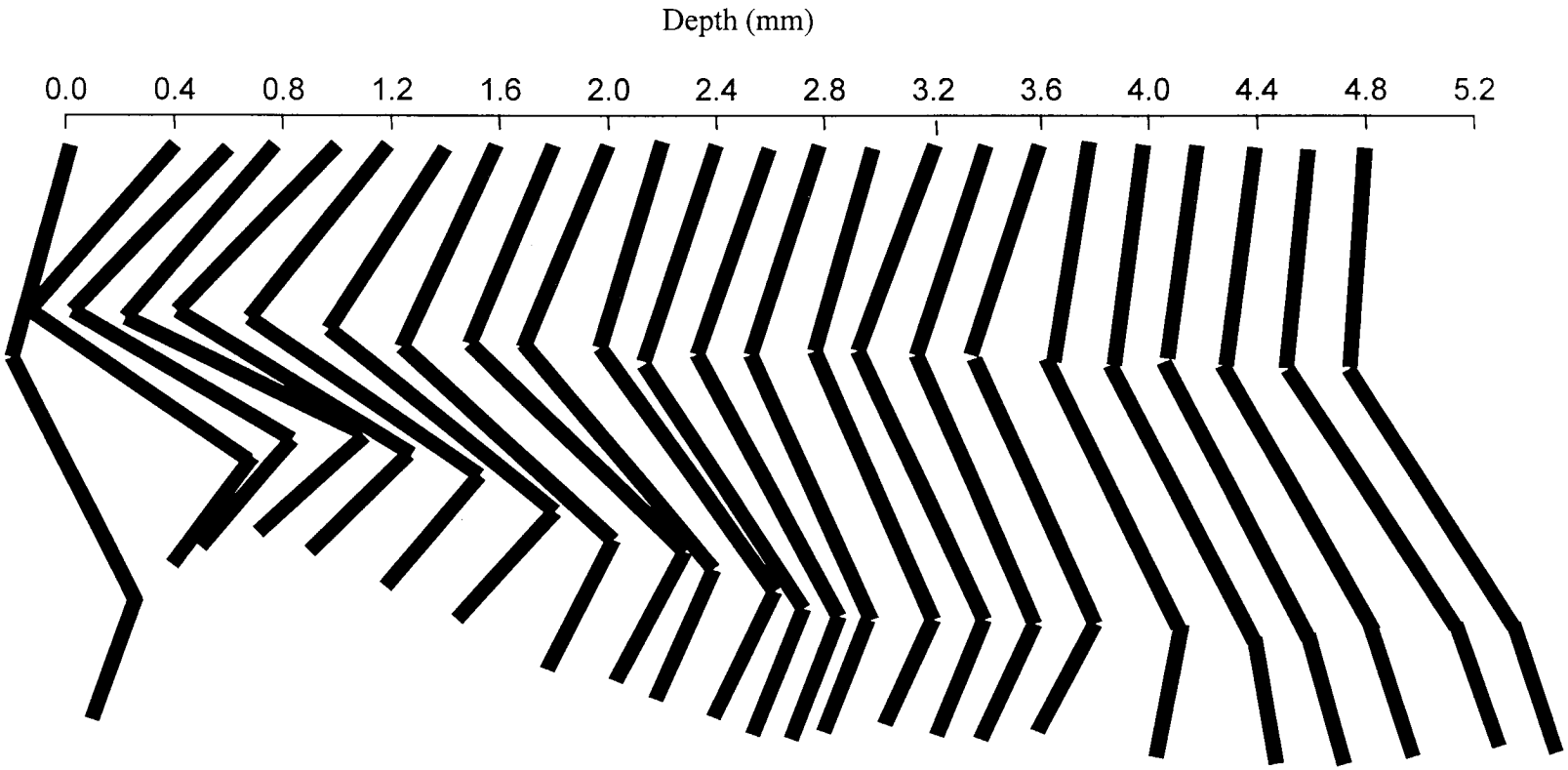
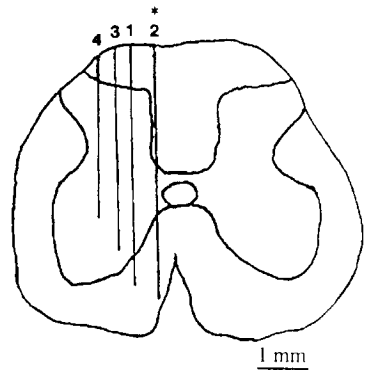


Figure 1

MS#77, Track#2, depth 0.4mm to 5.0mm, No.24-47



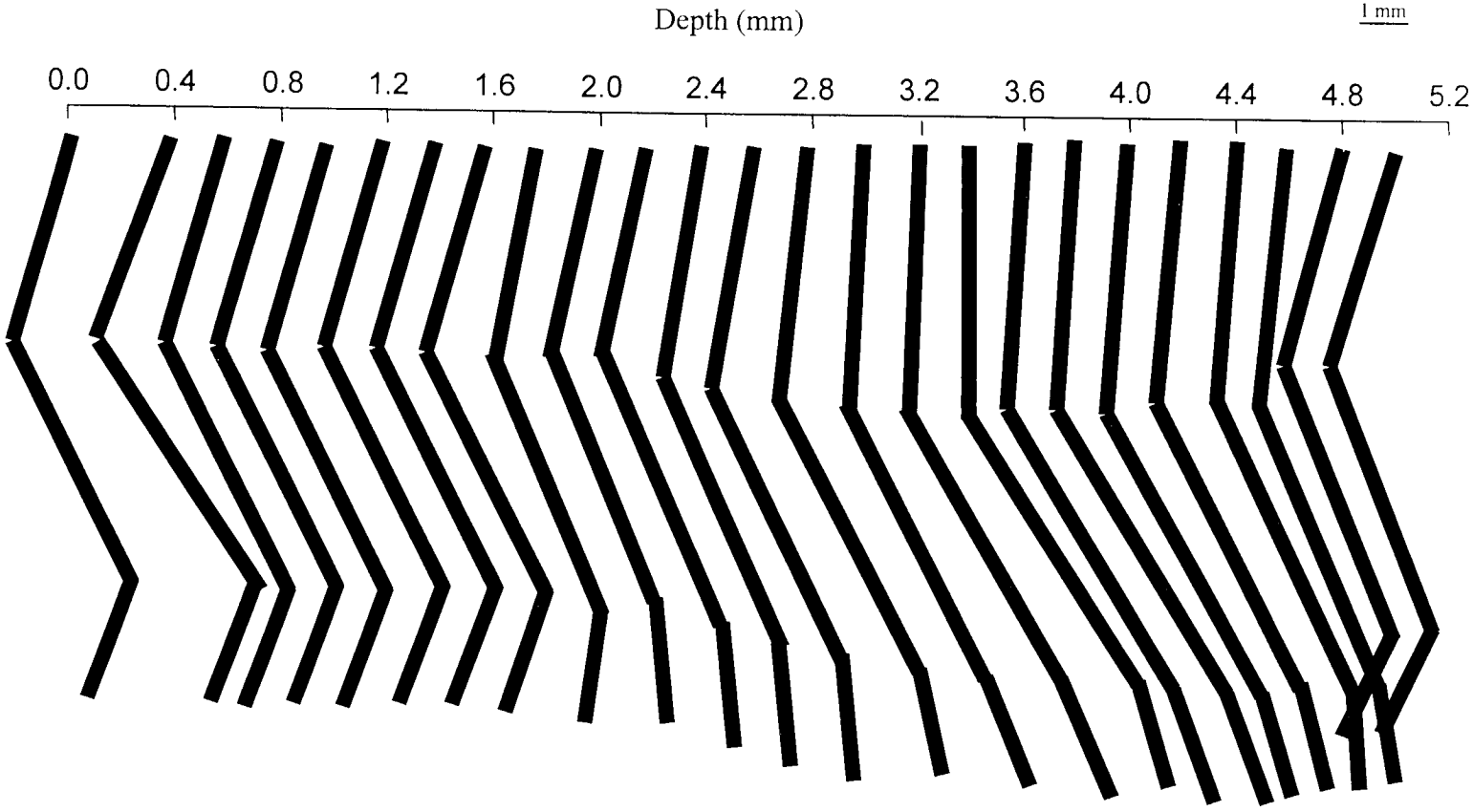
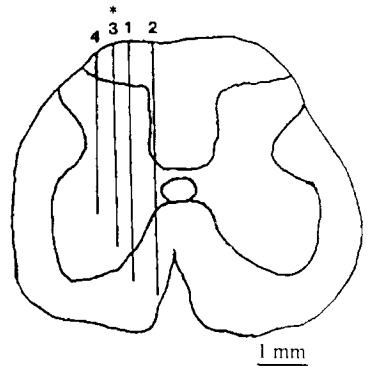


Figure 2

MS#77, Track#3, depth 0.4mm to 4.0mm, No.48-66



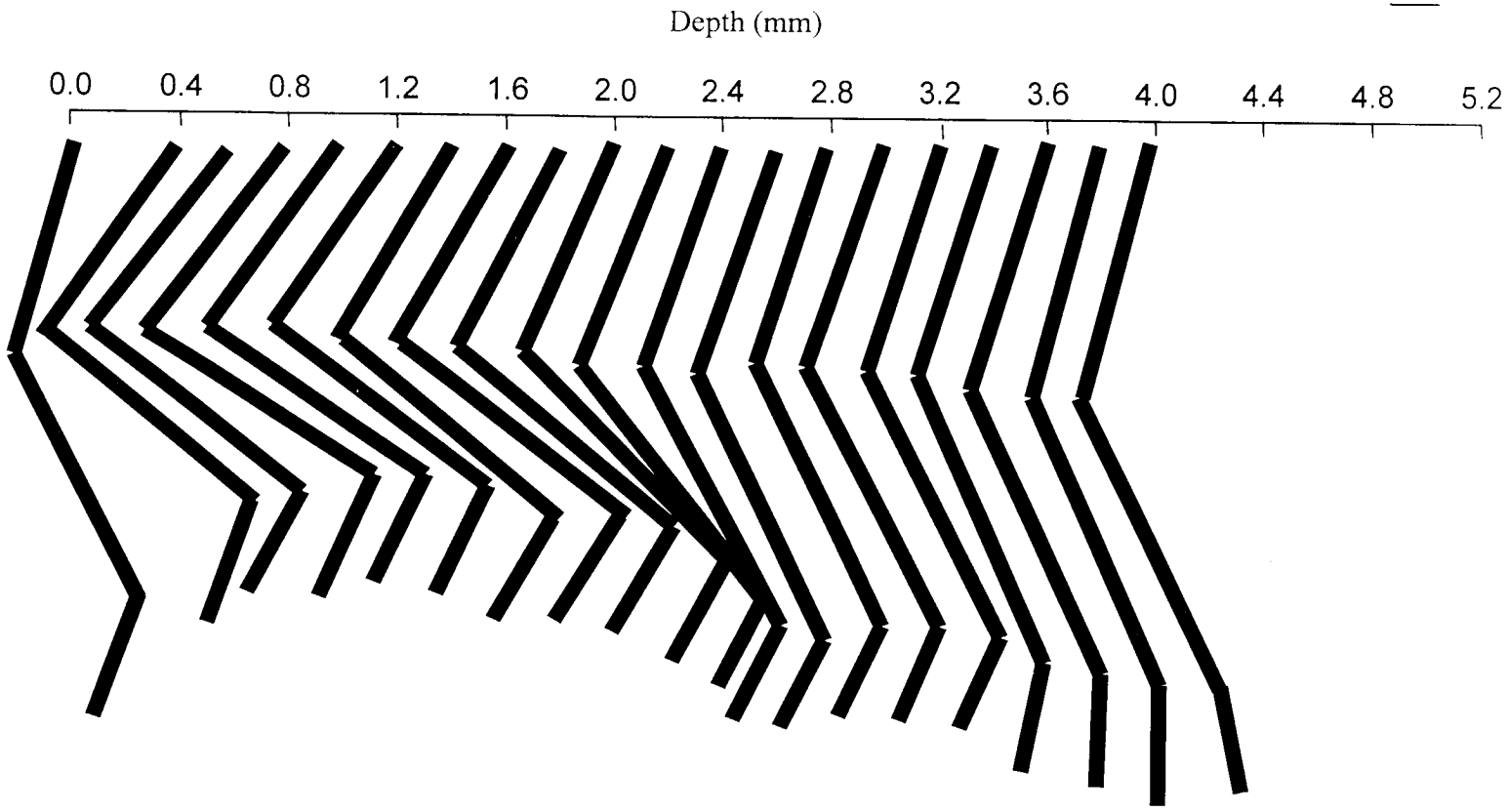
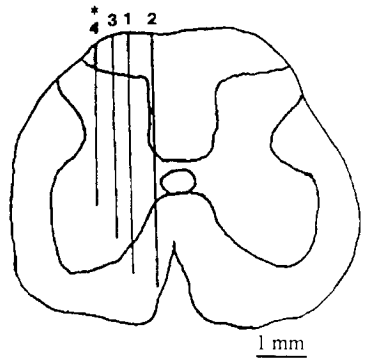


Figure 3

MS#77, Track#4, depth 0.4mm to 3.2mm, No.67-81



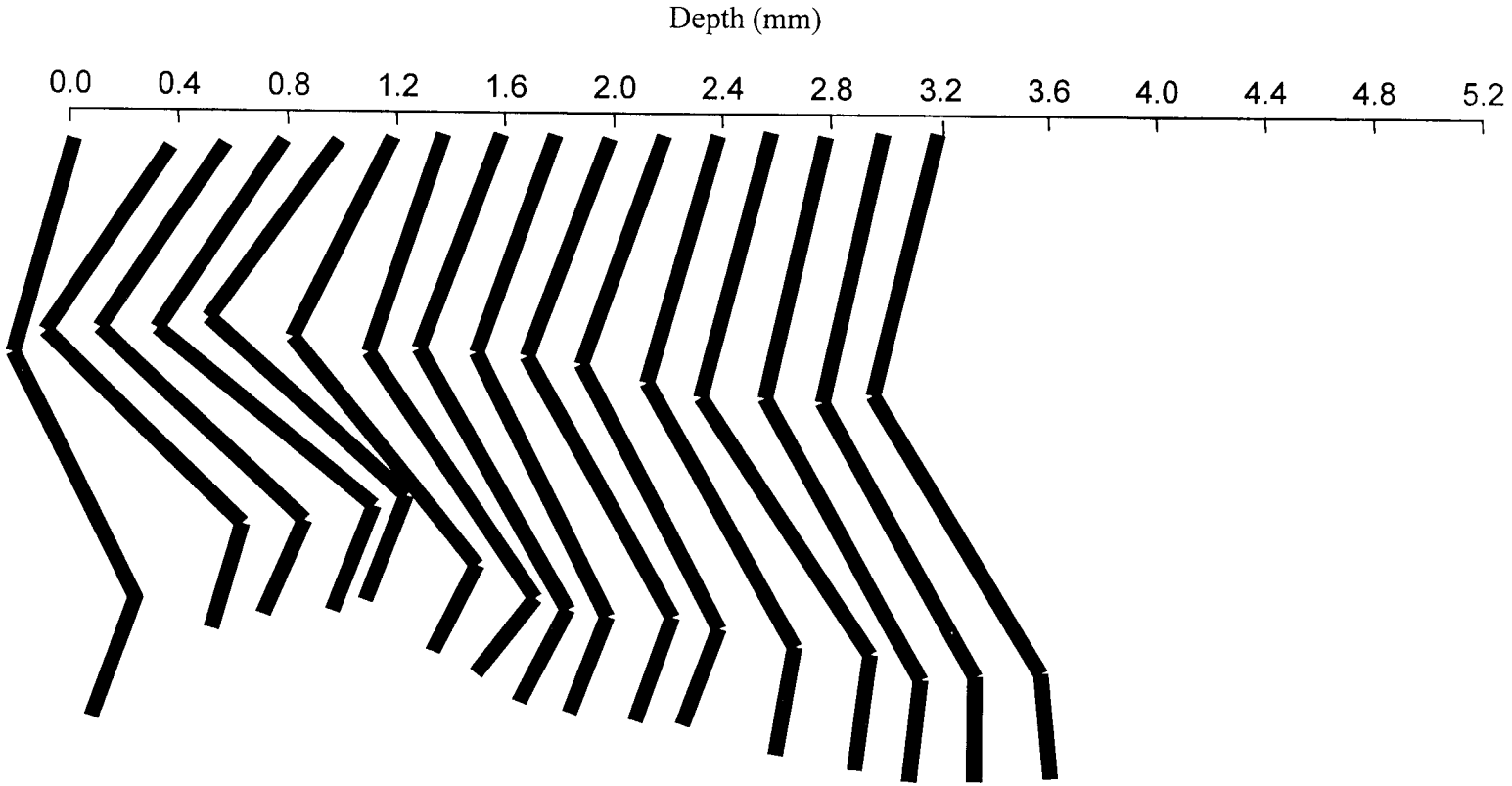


Figure 4

MS#77, Track#1, depth 0.4mm, movement#1

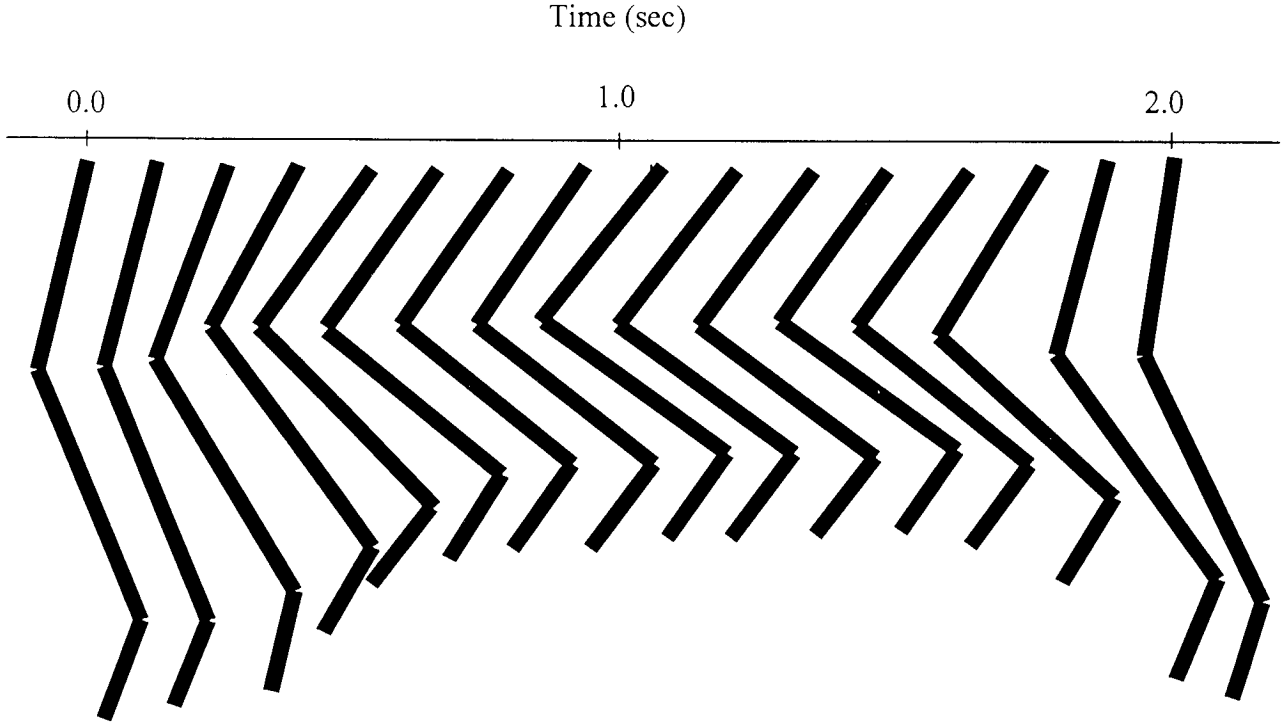


Figure 5

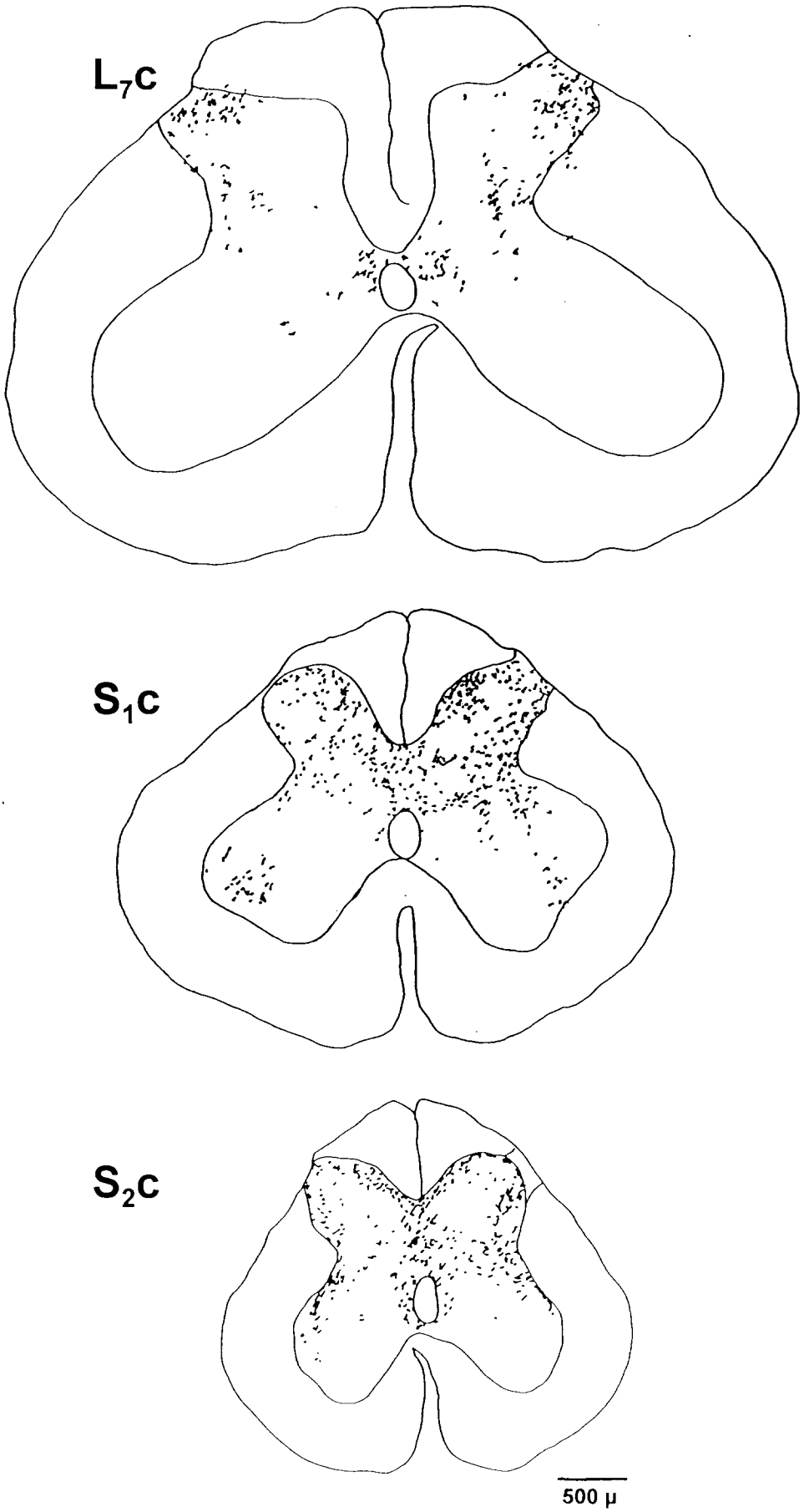


Figure 6